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TRANSFER OF F-LAC EPISOME TO PASTEURELLA PSEUDOTUBERCULOSIS

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Abstract

The F-*lac* episome was transferred from *Escherichia coli* to *Pasteurella pseudotuberculosis* at a frequency of 10^{-7} to 10^{-8}. The parent *P. pseudotuberculosis* fermented glycerol, rhamnose, salicin, and xylose, produced urease, VW antigens, and glucose-6-phosphate dehydrogenase, was pigmented, pesticin-sensitive, and virulent, and had a calcium requirement at 37 C. Some of the *P. pseudotuberculosis* F-*lac* recipients were negative for all of these properties, but became positive for all of them again when the F-*lac* episome was lost. It appeared that the F-*lac* carried with it a genetic unit that could repress the expression of several seemingly unrelated properties, and that this repression was removed when the episome was lost.

I. INTRODUCTION

The transfer of the F-lac episome from Escherichia coli to Pasteurella pestis has been reported by Martin and Jacob.[1] This episome was transferred at a frequency of 10^{-3} and 10^{-4} and the lactose-positive recipients appeared to be typical P. pestis. Our work extends these findings to Pasteurella pseudotuberculosis and reports on the new observation that in some cases several properties of the recipient P. pseudotuberculosis are altered when the F-lac episome enters the cell.

II. MATERIALS AND METHODS

A. STRAINS USED

During an investigation on the growth of P. pestis on various synthetic agar media, we observed rare colonies growing at 26 C on media containing only agar, glucose, and salts. These colonies proved to be indistinguishable from P. pseudotuberculosis type I when tested for the properties listed in Table 1. Although the possibility of contamination could not positively be eliminated, the appearance of these "P. pseudotuberculosis-like" organisms in several cultures that were started at different times from uncontaminated cultures of P. pestis, as well as their erratic appearance in cultures of P. pestis after treatment with bacteriophage, strongly suggested that some of the P. pestis organisms had been converted to P. pseudotuberculosis.

The observation was not new. D'Herelle[2] hypothesized that an acute disease might change into a chronic disease by the action of bacteriophage, and Harvey[3] speculated that this hypothesis might apply to plague; i.e., P. pseudotuberculosis might be a plague bacillus living in symbiosis with a bacteriophage. Soviet scientists have been more positive on this subject. Bessonova[4] reported the spontaneous "transformation" of P. pestis into P. pseudotuberculosis and Zhukov-Verezhnikov[5] and Khukov-Verezhnikov et al.[6] claimed that bacteriophage can convert P. pestis into P. pseudotuberculosis.

The data reported in this paper were obtained with strains labelled P. pseudotuberculosis that were isolated from five different strains of P. pestis, either spontaneously or after treatment with bacteriophage. The strain used to pass the F-lac episome into P. pseudotuberculosis was E. coli K12 pro⁻ F-lac. This strain received the F-lac episome from Salmonella typhosa F-lac, which was provided to us by Dr. Stanley Falkow.

TABLE 1. DISTINGUISHING PROPERTIES BETWEEN P. PESTIS AND P. PSEUDOTUBERCULOSIS AND THE REPRESSION OF THESE PROPERTIES BY THE F-LAC EPISOME

Properties	P. pestis wild type (oceanic)	P. pseudotuberculosis Parent Type	P. pseudotuberculosis F-lac	P. pseudotuberculosis F⁻ (lac⁻)
Lactose	-	-	+	-
Glycerol	-	+	-	+
Rhamnose	-	+	-	+
Salicin	+	+	-	+
Xylose	+	+	-	+
Melibiose	-	+	+	+
[illegible]	-	+	-	+
Pesticin sensitivity	-	+	-	+
Pigmentation	±a/	±	-	±
VW antigens	±	±	-	±
Calcium requirement	±	±	-	±
G-6-PO_4 dehydrogenase	-	+	-	+
Phage sensitivity	+	-	-	-

a. ± indicates that these properties can be either + or -. They are + in fully virulent strains.

B. F-LAC TRANSFER TECHNIQUE

Cultures were shaken in Difco heart infusion broth at 26 C for P. pseudotuberculosis and at 37 C for E. coli. Approximately 10^9 cells of each type were mixed, incubated at 26 C for 2 hours, and plated at appropriate dilutions on the following selective medium. Bacto agar, 1.5%; $MgSO_4 \cdot 7H_2O$, 0.01%; $ZnSO_4$, 0.00001%; $MnSO_4 \cdot H_2O$, 0.00001%; NH_4Cl, 0.053%; $(NH_4)_2SO_4$, 0.053%; $FeSO_4 \cdot 7H_2O$, 0.01%; $Na_2S_2O_3 \cdot 5H_2O$, 0.0025M; KH_2PO_4 - Na_2HPO_4 buffer, pH 7.2, 0.01 M; lactose, 1.0%. P. pseudotuberculosis will not grow on this medium unless it receives the F-lac episome and thus can use the lactose.

C. ASSAY OF PROPERTIES

Production of acid in carbohydrates was determined by inoculating the test organism into Bacto nutrient broth containing 1% carbohydrates and 0.04% brom thymol blue. Urease was detected by inoculating the test organism into Bacto urea broth. Pigmentation (pig) was determined on hemin agar.[7] V and W antigens[8] were detected by use of a gel plate technique.[9] Pesticin was assayed as described by Brubaker and Surgalla.[10] The calcium requirement was determined by use of magnesium oxalate agar.[11] Bacteriophage sensitivity was measured at 26 C by spotting a drop of C16 phage[12] on a lawn of the test organism on Difco blood agar base (BAB). Glucose-6-phosphate dehydrogenase was assayed by the method of DeMoss,[10] with NADP (nicotinamide-adenine dinucleotide phosphate) substituted for NAD (nicotinamide-adenine dinucleotide).

III. RESULTS

Our initial results indicated that the F-lac episome could be transferred from E. coli K12 F-lac pro$^-$ to P. pseudotuberculosis at frequencies between 10^{-7} and 10^{-8}. Lactose-positive colonies appearing on the selective medium were picked and streaked out three times on the same medium and then identified as P. pseudotuberculosis by antigenic analysis in a gel plate. Each of the lactose-positive P. pseudotuberculosis strains spontaneously segregated lactose-negative colonies, presumably as a result of the spontaneous loss of the F-lac episome. If a lac$^-$ segregant was remated with the same E. coli F-lac strain or a P. pseudotuberculosis F-lac strain, the frequency of F-lac transfer was increased to approximately 10^{-4}.

The first indication that other properties of the cell may be changed when the F-lac episome is introduced into P. pseudotuberculosis was the observation that P. pseudotuberculosis F-lac had 70% lac$^+$ and 30% lac$^-$ colonies on Difco eosin methylene blue agar (EMB) and 70% pig$^-$ and 30% pig$^+$ colonies on hemin agar. To check on this apparent correlation, a 50:50 mixture of lac$^+$ and lac$^-$ cells from this strain was plated on EMB agar at a dilution that produced 140 colonies per plate. By replication of these colonies to hemin agar with a sterile velvet cloth, we demonstrated that all lac$^+$ colonies were pig$^-$ and all lac$^-$ colonies were pig$^+$. It appeared that the F-lac episome carried with it the ability to repress the expression of pigmentation, and when the episome was lost, the repression was removed.

To determine whether properties other than pigmentation were affected by the presence of the F-lac episome, 20 lac$^+$ and 20 lac$^-$ clones of P. pseudotuberculosis F-lac were tested for acid production in glucose, glycerol, lactose, maltose, mannitol, rhamnose, salicin, xylose, and melibiose for pesticin sensitivity and for urease production. All of these properties except lactose fermentation are positive in the parent P. pseudotuberculosis. The clones containing the F-lac episome were negative for glycerol, rhamnose, salicin, xylose, pesticin sensitivity, and urease production. When the F-lac episome was lost, the resulting lac$^-$ cells were positive again for all of these properties. Further testing of cells containing F-lac has revealed that V and W antigens, the calcium requirement, and production of glucose-6-phosphate dehydrogenase can be repressed by the presence of this episome. A summary of the properties involved is presented in Table 1.

After acceptance of the F-lac episome, the P. pseudotuberculosis strains initially showed 10 to 30% lac$^-$ segregants. Upon storage at 5 C on BAB under mineral oil for approximately 1 month, four of the original five strains of F-lac P. pseudotuberculosis became stably lac$^+$ and stably repressed as summarized in Table 1. The fifth strain continued to show 10 to 30% lac$^-$ segregants and showed no repression, i.e., it had the same properties as the parent P. pseudotuberculosis. Although the presence or absence of the F-lac episome did not alter the properties of this strain, we have demonstrated that it can pass its F-lac episome to a different strain and the recipient strain then may be repressed for all the properties previously mentioned.

One of the four repressed P. pseudotuberculosis strains showed, in addition to the properties listed in Table 1, an inability to synthesize tryptophan. The parent P. pseudotuberculosis has no amino acid requirements at 26 C, but this strain required tryptophan to grow if the F-lac episome was present and lost its tryptophan requirement when it lost the F-lac episome.

Since VW antigens and pigmentation are known requirements for virulence,[14] it was not surprising to find that virulent P. pseudotuberculosis lost its virulence when VW antigens and pigmentation were repressed. Whenever a lac$^+$ repressed virulent strain that was segregating lac$^-$ unrepressed cells was inoculated into guinea pigs, only lac$^-$ unrepressed organisms were isolated from the spleen of the animals that died.

IV. DISCUSSION

Our results can be interpreted by hypothesizing that the F-lac episome carries with it a genetic unit that can prevent several different properties from being expressed. This finding is analogous to the suggestion of Clark and Adelberg[18] that "Colicin I carries not only structural genes, which determine surface properties, but also a regulatory gene capable of repressing the expression of the structural genes." We do not know yet if the F-lac episome can cause the repression of all the diverse properties outlined by altering the permeability of the cell or by acting directly at the gene level.

The hypothesis that the F-lac episome may carry a regulatory gene that can prevent the expression of certain properties has a special interest because of the nature of those properties. Five of the properties repressed are distinguishing properties between P. pestis and P. pseudotuberculosis (Table 1 -- rhamnose, glycerol, urease, pesticin sensitivity, and glucose-6-phosphate dehydrogenase production). Thus, a repressed P. pseudotuberculosis cell could be identified by mistake as P. pestis if any of these five properties were used to distinguish the two species. Further investigation into the nature of the repression effect of the F-lac episome may disclose not only additional information about this episome but also may reveal information about the genetic differences between P. pestis and P. pseudotuberculosis.

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